The Responses to Light in the Earthworm, Pheretima Agrestis Goto and Hatai, with Special Reference to the Function of the Nervous System

PART OF A DISSERTATION SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES
OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

By

CHARLES DEWITT HOWELL

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THE RESPONSES TO LIGHT IN THE EARTHWORM, PHERETIMA AGRESTIS GOTO AND HATAI, WITH SPECIAL REFERENCE TO THE FUNCTION OF THE NERVOUS SYSTEM

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EIGHT FIGURES

INTRODUCTION

It is well-known that earthworms are negative in moderately strong and strong light (Hoffmeister, 1845; Darwin, 1881; Graber, 1883; Loeb, 1894; Hesse, 1896; Parker and Arkin, '01; Smith, '02; Adams, '03; Harper, '05; Holmes, '05; Mast, '11; Hess, '24; Nomura, '26; Prosser, '34), and positive in extremely weak light (Adams, '03; Hess, '24; and Prosser, '34). However, practically all observations on responses to light in earthworms have been made on members of the two common genera, Eisenia and Lumbricus, both in the family Lumbricidae of the Oligochaeta. Only a limited study has been made of the responses to light in Pheretima (Harper, '05), therefore the discovery of Pheretima agrestis on the campus of the Johns Hopkins University afforded an opportunity to extend these observations in detail not only to a new species, but to a new family of Oligochaeta, the Megascolecidae.

[1] This paper is based on a dissertation submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Johns Hopkins University. The author takes this opportunity to thank Prof. S. O. Mast for his invaluable assistance in the course of this investigation and in the preparation of this paper.

I. RESPONSES OF PHERETIMA AGRESTIS IN LATERAL ILLUMINATION OF DIFFERENT INTENSITIES

MATERIALS AND METHODS

Pheretima agrestis Goto and Hatai[2] was used exclusively in this investigation. The first report of the occurrence of this genus (formely Perichaeta) in America was made by Garman in 1888. Since that time several species have been found in or near greenhouses in various localities. Pheretima agrestis is commonly found in Japan, but was introduced into this country with botanical products imported probably 15 to 20 years ago. It is distinguished from the common American species by its iridescent grayish color, by possessing numerous (often as many as 100) setae in each segment, by its clitellum which completely surrounds the body in segments 14 to 16, by its retractile proboscis which protrudes in front of the prostomium, by its tough epidermis and firm body wall, and by its violent snake-like squirming when disturbed.

Specimens were collected and kept in leaf-mold in large containers in a cool place the year round. No differences were observed in the responses of specimens freshly collected and those which had been kept for a whole year in leaf-mold in the laboratory.

All observations on their responses to light were made in a basement darkroom. The apparatus used consisted of a horizontal swivel table with a glass top 35 cm. square; a lamp transmitting only dim red light with a wave length greater than 650 mμ. and suspended directly over the center of the table at such a distance that the light produced was just sufficient to make objects on the table faintly visible; a tungsten lamp mounted in a light-tight shaft with an aperture 37 mm. square facing the swivel table; Wratten neutral tint light filters which could be inserted in a holder in front of the aperture in the shaft; a shutter which could be used

[2] The author is indebted to Prof. H. W. Olson of State Teachers College, Mansfield, Pa., for the identification of the species used.

to close the aperture; two black screens, each with an aperture the same size as that in the shaft and in line with it, and placed between the shaft and the swivel table to cut out reflected light and to produce a horizontal beam of parallel rays of light crossing and in contact with the center of the swivel table.

The observations were made as follows: All lights in the darkroom were extinguished excepting the red lamp over the table. The light in the shaft was then turned on, the shutter closed, and a specimen placed on moist paper towelling on the swivel table, with its long axis perpendicular to the beam of light. It was left until it moved forward steadily and slowly. The movable glass top was then adjusted so that the anterior third of the body was in position to be illuminated by the beam of light. Then, when the specimen extended its body straight forward, the shutter was opened and the effect of the light observed.

If the specimen turned toward the light the response was recorded as positive, if it turned away from the light the response was recorded as negative, but if it moved forward without turning, no response was recorded and the shutter was closed and another trial given after a short interval.

In intensities so low that the worm could not be seen the following method was used: The specimen was put into a straight runway made of two pieces of glass covered by a third on the swivel table. The table was then turned so that the long axis of the runway was perpendicular to the beam of light from the shaft. The red light was turned off and the shutter opened. After several minutes had elapsed, the red light was turned on and the position of the worm noted. If it was in a sector 8.6° on either side of the projected axis of the runway as measured from the point of exit from the runway, it was considered to have failed to respond. If it was nearer the source of light than this, the response was recorded as positive, if further from the source, as negative.

Thus two methods were used in observing the response to light, one in which the position of the worm was ascertained

after it had crossed the beam of light (used in weak illumination), and another in which the first turn given following exposure to light was ascertained (used in strong illumination).

Twenty specimens were tested in each of ten different intensities ranging from 21.5 m.c. to 0.000097 m.c. and their responses recorded. The results obtained are presented in table 1 and figure 1.

TABLE 1

Relation between the intensity of lateral illumination and the percentage of negative responses in Pheretima agrestis. Each specimen given ten trials

INTENSITY OF ILLUMINATION IN METER-CANDLES	NUMBER OF SPECIMENS USED	PERCENTAGE OF NEGATIVE FIRST TURNS AFTER EXPOSURE IN LIGHT	PERCENTAGE OF NEGATIVE ORIENTATIONS WHEN CROSSING BEAM OF LIGHT
21.5	20	99.5 ± 0.33	..
10.8	20	98.5 ± 0.53	..
2.15	20	93.5 ± 1.19	..
1.08	20	..	100.0 ± 0.00
0.215	20	90.5 ± 1.29	..
0.0226	20	..	95.0 ± 0.87
0.00265	20	..	62.5 ± 1.70
0.00108	13	..	60.0 ± 2.18
0.00071	20	..	48.9 ± 2.69
0.000097	20	..	52.6 ± 2.19

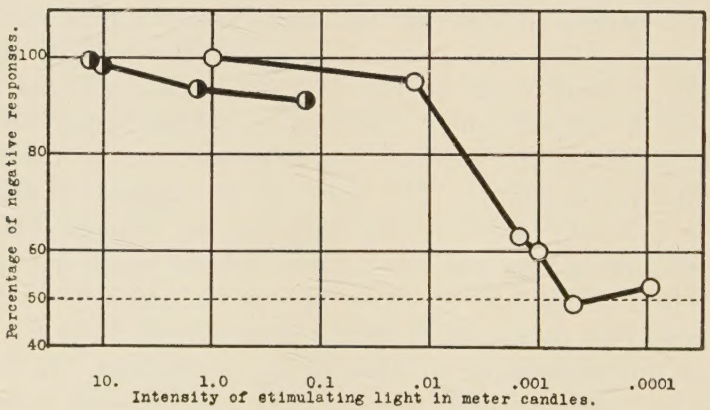

Fig. 1 Relation between the intensity of lateral illumination and the percentage of negative responses in Pheretima agrestis. ◑, calculated from the first turn given following each exposure; ○, calculated from the position of specimens after crossing the beam of light.

RESULTS

Table 1 and figure 1 show that the specimens were negative in 0.00108 m.c. and higher intensities, that they responded positively as often as negatively in lower intensities, and that they were more strongly negative in high than in low intensities.

Table 1 also shows that a higher percentage of negative responses was obtained if the nature of the response was ascertained by referring to the position of a specimen after crossing the beam of light, than if it was ascertained by referring to the direction of the first turn after exposure to light.

It is concluded from these results that Pheretima agrestis is negative in all intensities of light to which it responds, that it is more strongly negative in strong than in weak light, and that it does not respond to extremely weak light.

DISCUSSION

The responses of Pheretima agrestis in high intensities are in accord with the responses observed in Lumbricus and Eisenia, but the responses observed in low intensities are not. Adams ('03) found that Eisenia is positive in 70% of its responses to light of 0.0011 m.c., and Prosser ('34) that it is positive in 89.3% of its responses in light of 0.09 m.c. Hess ('24) found that Lumbricus is positive in 56.2% of its responses in 0.00118 m.c. The reason for the difference between Eisenia and Lumbricus, and Pheretima is not known. However, Adams points out that Eisenia appears on the surface of the earth at night, and postulates that this is due to the positive response in extremely weak light. Lumbricus also appears on the surface at night, but repeated observations on Pheretima over a period of 2 years indicate that it does not come to the surface at night. Either the fact that Pheretima is negative to weak light, or the fact that it is not positive to extremely weak light may account, at least in part, for this.

The fact that Pheretima appears to be more strongly negative if the nature of the response is ascertained by observing the position after it has crossed the beam of light than if it is

ascertained by observing the first turn after exposure is readily explained by the fact that, if a specimen is permitted to crawl across the beam of light it is continuously exposed to it and therefore has repeated opportunity to correct chance turns toward the light, and the fact that, if the first turn is recorded as the response, no opportunity is afforded to correct a chance first turn toward the light.

II. EFFECT OF ENVIRONMENTAL FACTORS ON THE RESPONSE TO LIGHT IN PHERETIMA AGRESTIS

INTRODUCTION

The following conditions decrease the responsiveness of various earthworms to light: conjugation (Hoffmeister, 1845; Darwin, 1881), feeding or dragging leaves (Darwin, 1881), close contact on all sides (Smith, '02), excessive mechanical stimulation (Hess, '24, et al.), exposure to strong light (Hess, '24), injury to the nervous system (Hess, '24; Nomura, '26 to '30; Prosser, '34), depressant drugs and exposure to low temperatures (Prosser, '34). They are less responsive also in colder seasons of the year (Nomura, '28 a), when the body is contracted (Holmes, '05; Harper, '09; et al.), and when sexually mature (Hess, '24). Jennings ('06) and Mast ('11) maintain that the response to light is affected by the physiological state of the organism.

The following three factors were selected and their effects on the responses to light in Pheretima studied: adaptation in light, temperature, and excessive mechanical stimulation.

1. LIGHT

Worms were adapted in different intensities of light as follows: A number of flat-bottomed, bowl-shaped containers, 30 cm. high and 38 cm. in diameter at the top were constructed of glazed white cardboard. Five to seven Petri dishes, each containing one worm lying on moist filter paper, were placed in a circle on the bottom of each of these containers. An electric lamp was then suspended over each container and left as long as desired. The sides of each bowl were curved

to reflect the incident rays of light toward the center of the bottom of the bowl so that the intensity of lateral illumination falling on the specimens in the Petri dishes was approximately half the overhead intensity. The temperature of the containers ranged from 21° to 24°.

Specimens used were first dark-adapted for 12 hours at room temperature (20° to 22°) and then adapted at a given

TABLE 2

The effect of adaptation in light on the percentage of negative responses to light in Pheretima agrestis. Percentages obtained in 0.108 m.c. are based on responses of ten worms; those in 2.15 m.c. are based on responses of twenty-five worms. Each worm was given ten trials. m.c., meter-candles

LUMINOUS INTENSITY IN METER-CANDLES IN WHICH SPECIMENS WERE ADAPTED	INTENSITY OF LATERAL ILLUMINATION IN WHICH SPECIMENS WERE TESTED			
	0.108 M.C.		2.15 M.C.	
	Percentage of negative responses	Percentage reduction in negative responses	Percentage of negative responses	Percentage reduction in negative responses
0 (Dark-adapted)	91.0 ± 1.51	0.0	93.6 ± 0.99	0.0
54	..	..	92.0 ± 3.00	1.71 ± 3.30
108	83.0 ± 2.86	8.79 ± 3.14	88.8 ± 1.33	5.22 ± 1.47
162	..	..	88.0 ± 1.45	5.98 ± 1.60
215	..	..	88.0 ± 1.47	5.98 ± 1.62
269	73.0 ± 2.70	19.8 ± 2.99	86.0 ± 1.59	8.12 ± 1.71
323	..	..	80.8 ± 1.60	13.7 ± 1.72
441	52.0 ± 2.07	42.8 ± 2.29	80.8 ± 1.51	13.7 ± 1.63
592	51.0 ± 2.21	43.9 ± 2.45	..	..
646	..	..	79.6 ± 1.82	15.0 ± 2.02

intensity for 1 hour. The worms were then tested in lateral illumination, some in 2.15 m.c. and others in 0.108 m.c.

The results obtained (table 2 and fig. 2) show that specimens adapted in light were less strongly negative than dark-adapted specimens, and that as the intensity of the light in which they were adapted increased the degree of negativity decreased to a minimum and then remained constant.

They also show that the effect produced by adaptation in any given intensity was greater on responses in the lower intensity used (0.108 m.c.), that is, the magnitude of the effect

depended not only on the intensity of the light in which the worms were tested, but also on the intensity of the light in which they were exposed before being tested.

They show, furthermore, that the worms failed to respond to 0.108 m.c. after adaptation in 441 and 592 m.c.; and that specimens which were adapted in light of a given intensity were still negative in lateral illumination of that and even much lower intensities. Since dark-adapted specimens

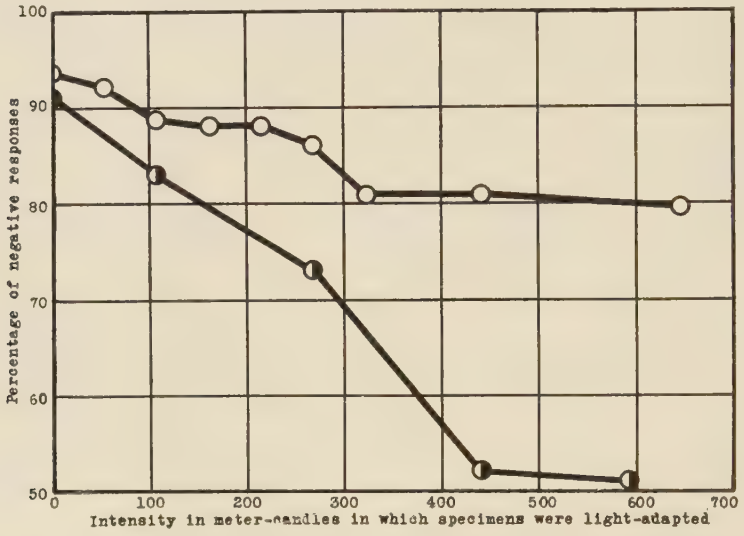

Fig. 2 The effect of adaptation in light of different intensities on the percentage of negative responses in lateral illumination in Pheretima agrestis. ◑, responses in 0.108 m.c.; ○, responses in 2.15 m.c.

normally respond to intensities as low as 0.001 m.c., the fact that adaptation in strong light produced a failure to respond to 0.108 m.c. proves that light-adaptation raises the threshold-intensity of light to which Pheretima responds. However, since the results presented in this paragraph also show that specimens still remained negative in response to light of the same and even much lower intensities than that in which they were adapted, it is concluded that adaptation of Phere-

tima in overhead illumination has a relatively small effect on the responses to lateral illumination.

Hess ('24) found that if specimens of Lumbricus which are light-adapted in illumination of 720 m.c. from above and 225 m.c. from below, they are positive in lateral illumination of a higher intensity than dark-adapted specimens. It is a direct corollary of this that light-adaptation raises the threshold-intensity of light to which Lumbricus is negative. In this respect, light-adaptation produces the same effect on Pheretima and on Lumbricus.

TABLE 3

The effect of temperature on the percentage of negative responses to lateral illumination in Pheretima agrestis. Each percentage is based on the responses of ten worms, each given ten trials

TEMPERATURE TO WHICH SPECIMENS WERE EXPOSED	INTENSITY OF LATERAL ILLUMINATION IN WHICH RESPONSES WERE TESTED			
	0.108 M.C.		2.15 M.C.	
	Percentage of negative responses	Number of failures to respond	Percentage of negative responses	Number of failures to respond
°C.				
15 ± 1.0	74.0 ± 1.40	19	89.0 ± 1.48	12
20 ± 1.5	90.0 ± 1.34	4	96.0 ± 1.04	0
26 ± 1.0	98.0 ± 0.85	2	99.0 ± 0.64	0
29 ± 1.0	92.0 ± 1.28	4	95.0 ± 1.07	2

2. TEMPERATURE

Worms were dark-adapted for 12 hours at temperatures ranging from 15° to 29°. Half of those adapted at each temperature were tested in lateral illumination of 2.15 m.c., the rest in 0.108 m.c. at room temperature (20° to 22°).

The results obtained (table 3, and fig. 3) show that as the temperature increased the degree of negativity increased to a maximum at 26° and then decreased slightly. They show also that the number of failures to respond decreased to a minimum at 26° and then increased slightly, and that specimens were more strongly negative and responded more frequently in strong than in weak light. Thus the degree of negativity and the number of failures to respond depend on

the temperature at which specimens are adapted and also on the intensity of light in which they are tested.

In the course of this work it was observed that specimens exposed to 15° were very sluggish and that as the temperature increased they became more active.

These results are essentially in accord with the results obtained by Prosser on Eisenia ('34), however, he gave no explanation of the decrease in negativity above 26°. It has

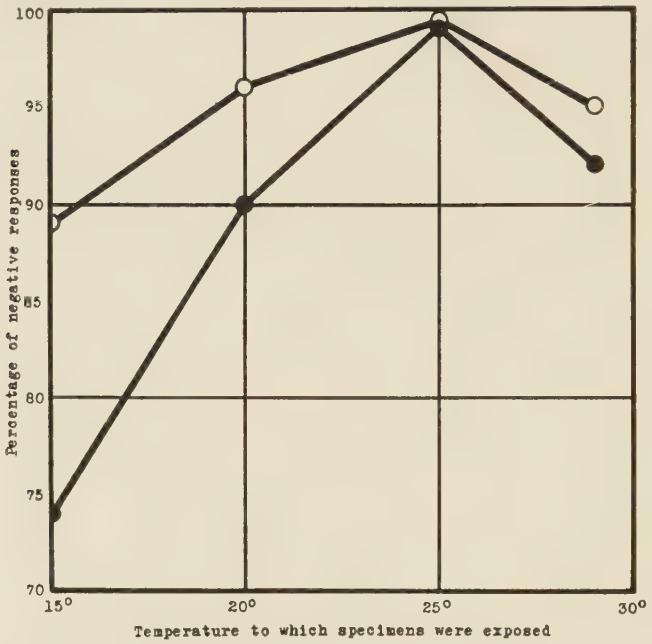

Fig. 3 The effect of temperature on the percentage of negative responses in lateral illumination in Pheretima agrestis. ○, responses in 2.15 m.c.; ●, responses in 0.108 m.c.

been observed in this work that temperatures above 30° produce abnormal activities, and it is known (Smith, '03) that 35° to 40° is lethal to earthworms. It is probable that temperatures above 26° are injurious and produce responses which are specific for injurious stimuli and which inhibit responses to light. If this is true it accounts for the decrease in negativity as the temperature is raised above 26°.

3. EXCESSIVE MECHANICAL STIMULATION

Specimens which had been tested in lateral illumination of 2.15 m.c. in the usual way were used for these experiments. They were dark-adapted for 12 hours and then stimulated by prodding the posterior ends intermittently with a blunt dissecting needle for 1 minute. Immediately following this they were tested in lateral illumination of 2.15 m.c.

Prodding at first produces a brief lengthwise contraction of the whole body, after which worms move forward rapidly in a straight line. However, after repeated prodding, specimens frequently fail to contract after each stimulus, but move forward more rapidly as the stimulation is continued, till a maximum is reached, and then continue to move for some time after the stimulation has ceased. Exposure to lateral illumination was made after prodding had ceased but while the specimens were still moving forward rapidly. The results obtained are presented in the following paragraph.

The results show that on specimens which had not been prodded 90.5% of the exposures resulted in negative responses, 9.5% in positive responses, and none in failures to respond. But they show also, that on specimens which had been prodded only 67.7% of the exposures resulted in negative responses, whereas 18.5% resulted in positive responses, and 13.8% in failures to respond.

It is concluded from these results that excessive mechanical stimulation, like exposure to high temperature, decreases the negativity and the responsiveness to light, and that this effect results from the production of responses which are specific for injurious stimuli and which inhibit the response to light.

III. SENSITIVITY OF THE VARIOUS REGIONS OF THE BODY IN PHERETIMA AGRESTIS

A modification of Patten's apparatus (Patten, '17; Hess, '24) for localized stimulation with a small area of light was used to ascertain the sensitivity to light of the various regions of the body. This apparatus consists essentially of the body-

tube of a microscope with screens in it, a low power objective, and a flashlight lamp in the place of an ocular. The light from the lamp is concentrated in a small spot by the objective.

Owing to low illumination, difficulties were encountered in identifying the segments which were illuminated by the spot of light and in seeing the response to light. The results obtained show, however, that the order of sensitivity of the surface of the body is as follows: anterior four to five segments > posterior two to three segments > segments between these regions. The dorsal surface is more sensitive than the ventral surface of the body, excepting in the anterior three segments where the sensitivity of the dorsal side is equal to that of the ventral side. The second and third segments appear to be equally sensitive and are more sensitive than the prostomium, while the prostomium is more sensitive than the remainder of the first segment. The proboscis is not sensitive to light.

These conclusions are supported by the results obtained after various segments of the anterior end of the body had been removed (table 4). These results show that the removal of the segments of the anterior end caused a reduction in negativity which was proportional to the number of segments which were removed, and that the increments in reduction in negativity were: 0.36 for the first segment, 6.39 for the second, and 7.14 for the third. This indicates that the second and third segments are approximately equal in sensitivity and are more sensitive than the first segment.

Hess obtained similar results on Lumbricus, but found that the order of sensitivity of the segments of the anterior end is: Prostomium > remainder of the first segment > second segment > third segment > fourth segment. In respect to distribution of sensitivity, it is therefore obvious that Pheretima agrestis differs from Lumbricus. This difference is correlated with the fact that the first segment and the prostomium are usually found in an almost completely introverted position in Pheretima, but never in Lumbricus, and the fact that

the portions of the anterior end of the body which are continuously subject to exposure are the most sensitive.

The end organs for sensitivity to light in Lumbricus are single cells distributed throughout the epidermis and known as Lichtzellen or photoreceptors. They are more abundant in the more sensitive than in the less sensitive regions of the body (Hesse, 1896; Hess, '25 b), and are absent in the region

TABLE 4

The effect of removing various portions of the anterior end on the percentage of negative responses to lateral illumination in Pheretima agrestis.
m.c., meter-candles

PART REMOVED	LUMINOUS INTENSITY IN WHICH SPECIMENS WERE TESTED (IN M.C.)	PERCENTAGE OF NEGATIVE RESPONSES		PERCENTAGE CHANGE IN NEGATIVITY
		Before operation	After operation (average for 2 successive days)	
First segment including the prostomium	0.145	96.2 ± 1.82	95.7 ± 1.98	−0.57 ± 2.20
	1.45	98.0 ± 1.60	97.5 ± 2.05	−0.51 ± 2.22
	14.0	98.0 ± 1.54	98.0 ± 2.01	−0.00 ± 2.01
First two segments	0.145	96.2 ± 1.82	80.1 ± 2.98	−16.8 ± 3.29
	1.45	98.0 ± 1.60	95.6 ± 2.59	−2.4 ± 2.81
	14.0	98.0 ± 1.54	99.0 ± 2.14	+1.0 ± 2.25
First three segments without the brain	0.145	96.2 ± 1.82	75.0 ± 2.92	−22.8 ± 3.24
	1.45	98.0 ± 1.60	81.2 ± 2.89	−17.1 ± 1.89
	14.0	98.0 ± 1.54	96.2 ± 2.58	−1.8 ± 1.67
First three segments including the brain	0.145	91.1 ± 1.60	18.2 ± 2.54	−80.0 ± 2.72
	1.45	96.1 ± 1.52	20.9 ± 2.34	−78.3 ± 2.62
	14.0	96.9 ± 1.30	64.0 ± 2.20	−33.9 ± 2.44

which is insensitive to light (Hess, '25 b). Cells similar to the photoreceptors of Lumbricus were found in the epidermis of Pheretima. They will be considered in a subsequent paper.

IV. RESPONSES TO LIGHT IN PHERETIMA AGRESTIS AFTER VARIOUS OPERATIONS ON THE NERVOUS SYSTEM

1. THE NERVOUS SYSTEM OF PHERETIMA AGRESTIS

The nervous system of Pheretima agrestis differs in a number of respects from that in Lumbricus terrestris, therefore a brief description of it will be given here. The brain

lies beneath the groove between segments three and four, and is connected with the ventral nerve cord by means of a pair of heavy circumpharyngeal connectives (fig. 4). From five to nine cerebral nerves arise from either side of the brain where it joins the circumpharyngeal connective. These nerves are analogous to the pairs of cerebral nerves in Lumbricus (Hess, '24, '25 a) in that they innervate the anterior end of the buccal cavity (including the proboscis), and the prostomium. As in Lumbricus, a large nerve branches from the middle of each circumpharyngeal connective and

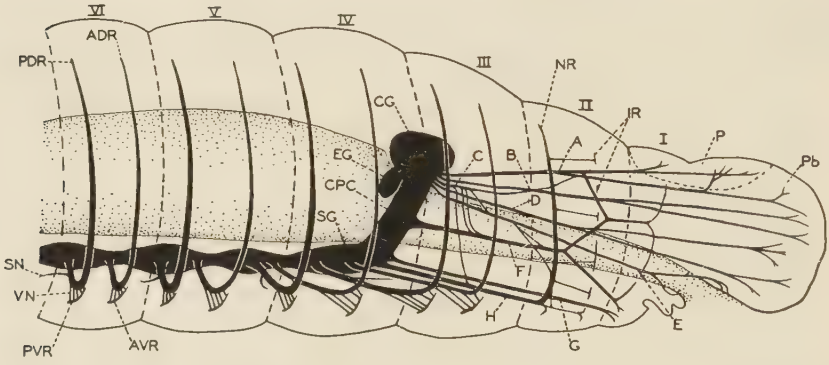

Fig. 4 Sketch of nervous system of the anterior end of the right side of the earthworm, Pheretima agrestis. A to E, cerebral nerves; ADR, anterior dorsal nerve ring; AVR, anterior ventral nerve ring; CG, cerebral ganglion (brain); CPC, circumpharyngeal connective; EG, enteric ganglion; F, nerve to lateral portion of first segment; G, nerve supplying ventral portion of first segment; H nerve supplying second segment; IR, incomplete nerve ring of second segment; NR, posterior nerve ring of second segment; P, prostomium; Pb, proboscis; PDR, posterior dorsal nerve ring; PVR, posterior ventral nerve ring; SG, subpharyngeal ganglion; SN, septal nerve; VN, ventral nerves. I-VI, segments one to six.

supplies the lateral portion of the first segment. The first pair of nerves that branches from the anterior end of the ventral nerve cord supplies the ventral portion of the first segment. The second pair supplies the second segment. The remaining segments of the body are usually supplied with but two pairs of nerves (the segmental nerves), a smaller anterior pair and a larger posterior pair. In Lumbricus each segment

is supplied with three pairs of segmental nerves. Each divides into a dorsal and ventral ramus. In Pheretima the corresponding dorsal rami unite in the mid-dorsal line, and the corresponding ventral rami unite in the mid-ventral line of the body. Thus, two complete nerve rings are formed by each pair of segmental nerves, a dorsal and a ventral ring. The segmental nerves of Lumbricus terrestris terminate near the midlines of the body without uniting.

2. OPERATIONS ON THE NERVOUS SYSTEM

Introduction

Graber (1883) observed that Lumbricus from which the anterior four to five segments including the brain had been removed definitely avoid light, although not so definitely as normal specimens. Similar observations were made by Loeb (1894) and Hesse (1896). Hess ('24) found that after removal of the anterior three segments and the brain, or after removal of the brain, Lumbricus is photopositive in lateral illumination of intensities in which it was photonegative before the operation. He found also that in specimens in which the ventral nerve cord had been cut, the portion of the body anterior to the cut is photonegative and the portion posterior to it is photopositive. He concluded that the brain is the controlling factor in producing negative orientation to light.

Nomura ('26) removed various parts of the body of Eisenia and came to the conclusion that the brain induces negative and the ventral nerve cord positive responses to light.

Prosser ('34) observed that specimens of Eisenia with the brain removed are positive to relatively low intensities of light and that as the intensity of light increases the degree of negativity increases. He maintains that the brain induces negative and the ventral nerve cord positive responses to light, and that in normal specimens the influence of the brain overcomes the influence of the cord, resulting in a negative response. Prosser found also, that severing the transverse commissure of the brain makes specimens as strongly posi-

tive as removing the entire brain, and that severing one circumpharyngeal connective makes them photopositive to stimulation of the unoperated side and photonegative to stimulation of the operated side of the body. He concluded that the normal dominance of the brain in producing negative orientation in Eisenia can be explained by assuming a crossing-over, in the transverse commissure of the brain, of impulses from the sensitive anterior end of the body.

Methods

Operations and observations similar to those made by Hess on Lumbricus and Prosser on Eisenia were made on Pheretima.

The specimens used were first dark-adapted for 12 hours at room temperature (19° to 22°) and tested in lateral illumination of 0.145, 1.45, and usually 14.0 m.c. as described in the first section of this paper. Each specimen was then anesthetized in a 0.3% solution of chloretone, and placed on a clean filter paper on a dissecting board beneath a dissecting binocular, after which an incision was made through the body wall above the brain. The incision was opened and the edges held apart by fine hooks on the ends of dissecting needles. The desired operation was then performed with a pair of fine scissors. The specimen was then rinsed in tap water, put into a Petri dish containing moist filter paper and left in the dark at room temperature.

Twelve hours after the operation and subsequently at intervals until recovery (except where otherwise stated) specimens were tested in lateral illumination. After each series of tests they were put into moist leaf-mold in Petri dishes and kept in the dark at room temperature until the next series of tests.

Each specimen was given twenty trials in each intensity unless it became inactive or unresponsive before the completion of the test. The results obtained are presented in the following pages.

Results

Tables 4 and 5 and figure 5 show that each of the operations performed decreased the degree of negativity of specimens in all intensities of light used. Table 5 shows that the order of effectiveness of the operations, beginning with the highest was: removal of the anterior three segments and the brain, separation of the two lobes of the brain, severance of both circumpharyngeal connectives near the ventral nerve cord, removal of the brain, severance of both circumpharyngeal connectives near the brain, severance of one connective near the brain, severance of one connective near the ventral nerve cord, and removal of the anterior three segments leaving the brain intact.

Table 4 shows that removing the segments of the anterior end caused only a slight reduction in negativity, which increased as the number of segments removed increased up to three (provided the brain was intact), but that if the brain was removed with the anterior three segments, the specimens became strongly positive in lateral illumination of intensities on which they were negative before the operation (viz., 0.145 and 1.45 m.c.).

The results obtained in the studies of the time required for recovery from the operations show that removal of the brain required 32 days, severance of both connectives required 10 days, separation of the lobes of the brain required 6.5 days, and severance of one connective required 4 days. It is evident that the time required for recovery increases as the amount of tissue injured or removed increases.

In the course of these studies it was observed that the time between the operations and functional recovery could in some cases be divided into three periods. This is illustrated in the upper graphs in figure 6, which show the changes in the percentage of negative responses at intervals after both circumpharyngeal connectives were severed near the ventral nerve cord. These graphs show that for 3.5 days after the operation there was a strongly positive response (first period). This

TABLE 5

Comparison of the effects of different operations on the nervous system on the percentage of negative responses to light in Pheretima agrestis.
Av., average

OPERATION	LUMINOUS INTENSITY IN METER-CANDLES	AVERAGE PERCENTAGE NEGATIVE RESPONSES BEFORE OPERATION	AVERAGE MINIMUM PERCENTAGE NEGATIVE RESPONSES AFTER OPERATION	AVERAGE PERCENTAGE REDUCTION IN NEGATIVITY
Anterior three segments and the brain removed	0.145	91.1±1.24	18.2±1.45	80.0±1.61
	1.45	96.1±0.58	20.9±2.52	78.3±2.62
	14.0	96.9±1.13	64.0±3.05	34.0±3.38
		Av. 94.7±0.98	Av. 34.3±2.34	Av. 64.0±2.54
Separation of the two lobes of the brain	0.145	90.0±1.65	21.5±1.69	76.1±1.87
	1.45	96.4±0.72	38.7±1.70	59.8±1.76
	14.0	98.0±0.85	69.3±1.98	29.3±2.06
		Av. 94.8±1.07	Av. 43.2±1.79	Av. 55.1±1.90
Both connectives severed near the ventral nerve cord	0.145	90.0±1.06	24.3±1.61	77.5±1.79
	1.45	95.4±1.19	30.9±1.45	67.6±1.52
	14.0	96.0±1.40	84.0±1.71	12.5±1.78
		Av. 93.8±1.22	Av. 46.4±1.59	Av. 52.5±1.70
Brain removed	0.145	92.0±0.85	23.0±1.02	75.0±1.11
	1.45	96.0±1.04	37.1±1.19	61.3±1.24
	14.0	97.0±0.98	88.1±1.41	9.2±1.47
		Av. 95.0±0.96	Av. 49.4±1.21	Av. 48.5±1.27
Both connectives severed near the brain	0.145	91.0±1.49	22.0±2.09	75.8±2.30
	1.45	94.0±1.42	59.0±2.92	37.6±3.32
	14.0	98.0±1.20	92.0±2.25	6.1±2.34
		Av. 94.3±1.37	Av. 57.7±2.42	Av. 39.8±2.65
One connective severed near the brain	0.145	91.1±1.04	46.3±1.71	49.2±1.90
	1.45	95.0±0.86	56.2±2.75	40.8±3.00
	14.0	99.0±0.47	81.2±1.91	18.0±1.93
		Av. 95.0±0.79	Av. 61.2±2.19	Av. 36.0±2.28
One connective severed near the ventral nerve cord	0.145	95.0±1.07	44.5±2.30	53.2±2.45
	1.45	98.0±0.85	62.5±2.03	36.2±2.07
	14.0	..	..	..
		Av. (96.5±0.96)	Av. (53.5±2.16)	Av. (44.7±2.26)
Anterior three segments removed, brain intact	0.145	96.2±1.15	75.0±1.68	22.1±1.74
	1.45	98.0±1.20	81.2±1.91	17.1±1.97
	14.0	98.0±0.85	96.2±1.15	1.8±1.79
		Av. 97.4±1.07	Av. 84.2±1.58	Av. 13.7±1.83

was followed by 3 days in which there was a rapid increase in the degree of negativity (second period), which in turn was followed by a much slower increase in negativity terminating

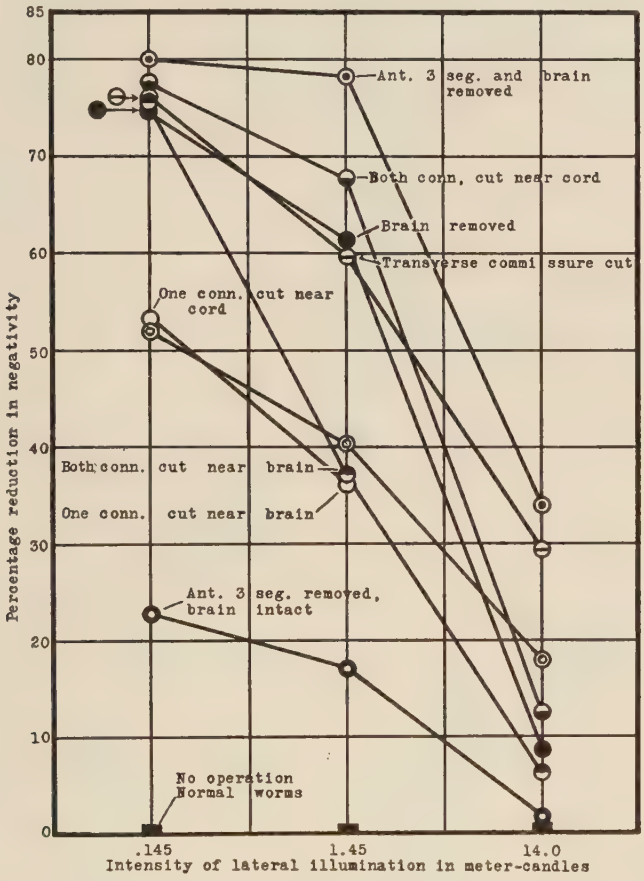

Fig. 5 The effects of different operations on the nervous system on the percentage of negative responses in lateral illumination in Pheretima agrestis.

in the normal response to light (third period). After operations which required a shorter time for complete recovery, the first and third periods are less pronounced or entirely absent.

From the results presented in table 5 and figure 5 it is obvious that separating the lobes of the brain had the same effect as removing the entire brain. However, it remained to be shown whether the effect observed was the result of severance of the fibers of the transverse commissure of the brain or of injury to other tissues in the brain.

If the results obtained were caused by severing the transverse commissure, functional recovery should parallel the

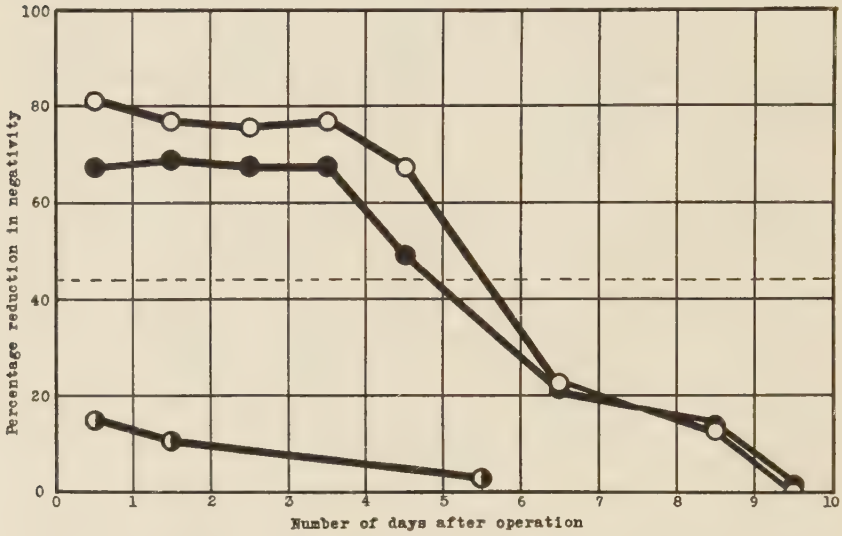

Fig. 6 The course of recovery of normal negativity in lateral illumination after severing both circumpharyngeal connectives near the ventral nerve cord. Responses in light of 0.145 m.c., ○; of 1.45 m.c., ●; of 14.0 m.c., ◑. Points above the broken line represent predominantly negative responses, those below, predominantly positive responses. Explanation in text.

regeneration of the fibers in the transverse commissure. To test this, histological studies and observations on the response to light were made on regenerating worms. The results obtained were as follows: Preparations of specimens killed 2 days after the operation showed that, at this time, the lobes of the brain were widely separated but connected by a band of regenerating neuroglia, and that few nerve fibers crossed from one lobe of the brain to the other (fig. 7 B). The aver-

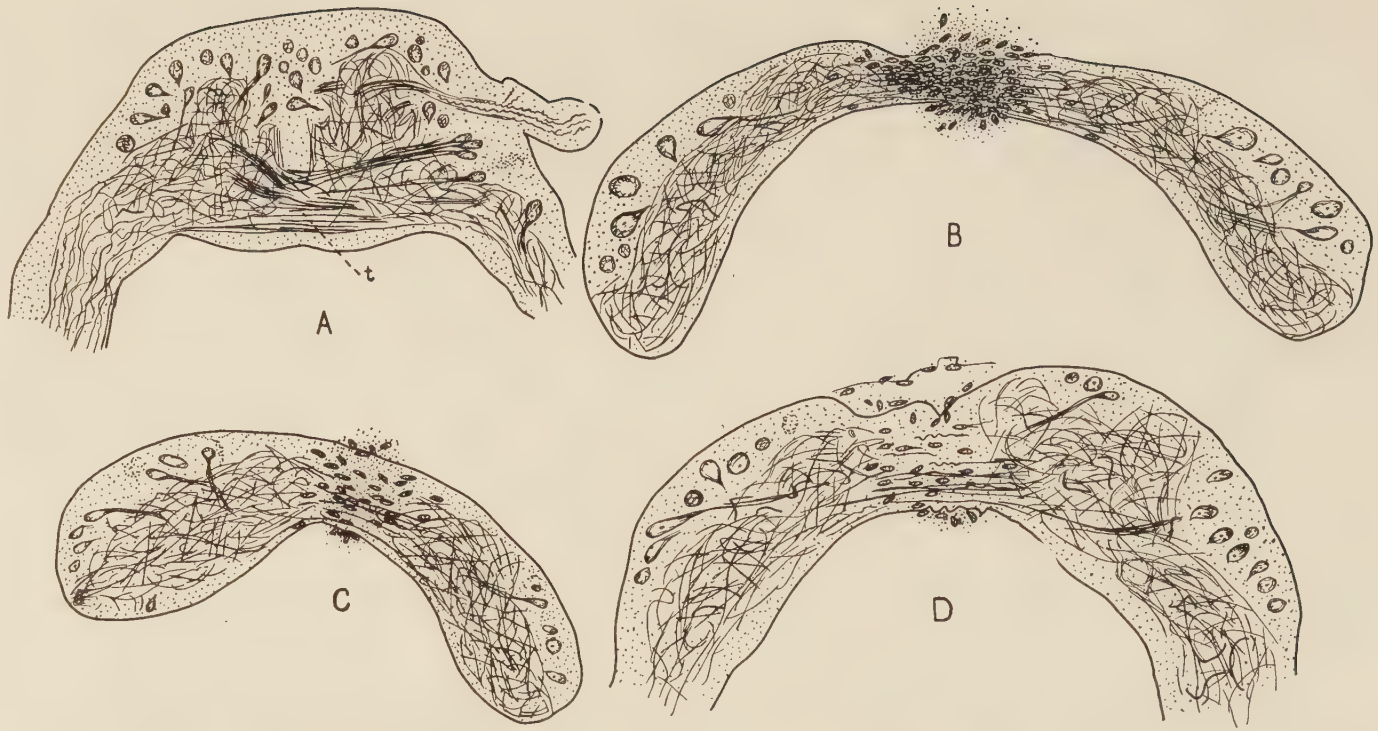

Fig. 7 Camera lucida drawings of normal and regenerating brains in Pheretima agrestis. A, normal brain, showing transverse commissure, t; B, C, D, progressive stages in regeneration of the transverse commissure after separating the two lobes of the brain. Further explanation in text.

age percentage of negativity of these specimens just before they were killed was 23% (range, 15 to 30%). Preparations of specimens killed 3.5 days after the operation showed that, at this time, the lobes of the brain were drawn more closely together than those of the specimens killed at 2 days, and that they were joined by from twelve to nineteen fibers (fig. 7 C). The average percentage of negativity of these specimens just before they were killed was 42% (range, 40 to 45%).

Furthermore, preparations of specimens killed 5.5 days after the operation showed that the lobes of the brain were very close together and were connected by a well-developed transverse commissure of at least twenty-three fibers (fig. 7 D). These specimens gave an average of 85% negative responses. Finally, preparations of specimens killed 7 days after the operation showed that the brain was practically normal in appearance. These same specimens were negative in 92% of their responses to light, and were therefore normal.

These results are in accord with those obtained by Prosser on Eisenia ('34). They show that there is a close correlation between the regeneration of nerve fibers in the transverse commissure and recovery of the normal response to light. However, they do not prove that regeneration of other tissues is not also involved in this recovery. The following experiment was therefore performed: The lobes of the brain were separated in four specimens. Three days later these specimens were tested and found negative in 62.5% of their responses to lateral illumination of 0.145 m.c. They were then anesthetized and the incision previously made in the body-wall was reopened. It was found that the lobes of the brain were still widely separated in three of these specimens, but were close together in the fourth. The region in which the regenerating fibers lay was then severed without chance of injury to the two lobes of the brain. Twenty-four hours later, these specimens were again tested and were found negative in 22.5% of their responses in 0.145 m.c. Five days later they were negative in 95% of their responses, and subsequent autopsy proved that the brain was then practically normal

and had a well-developed transverse commissure. From this experiment it is concluded that the great reduction in negativity produced by separating the two lobes of the brain is the result of severing the fibers of the transverse commissure and not of injury to the neuropile of the lobes of the brain.

The results obtained in observations on specimens with one connective severed near the ventral nerve cord, and on specimens with one connective severed near the brain show that after illumination of the operated side these specimens gave an average of 70.1 $\pm$ 2.7% negative responses, and that after illumination of the unoperated side, they gave only 45.9 $\pm$ 2.62% negative responses. Prosser obtained similar results on Eisenia.

Discussion

Hess (’24), as previously stated, found that removal of the cerebral ganglion in Lumbricus causes it to become definitely positive in intensities in which it previously had been negative. Nomura (’26) obtained the same results on Eisenia and concluded that the brain causes negative and the ventral nerve cord positive responses. Prosser (’34) shows, however, that Eisenia with the brain removed is positive in relatively weak light but that it becomes less strongly positive as the intensity increases. He concluded, therefore, that the reversal from negative to positive orientation following injury to the brain is not a true reversal but merely the result of an increase in the threshold intensity of light necessary for a predominance of negative responses.

Prosser’s conclusions are strongly supported by the fact presented above, that while Pheretima with the brain removed is positive in weak illumination, its positivity decreases to such an extent as the intensity increases that it becomes strongly negative (88.1% negative in 14.0 m.c.). This proves conclusively that the brain is not necessary for negative responses to light in this organism.

It is obvious that in order to produce negative orientation in lateral illumination, impulses must pass from the receptors

on the illuminated side to the muscles of the opposite side of the body and cause their contraction. Studies of the nervous system show that nerve fibers cross from one side of the body to the other in the brain and in the ganglia of the ventral nerve cord. This raises the question as to where the impulses arising from photic stimulation cross over.

It was demonstrated in preceding pages that normal specimens of Pheretima are negative in all intensities of light to which they respond, but that if the transverse commissure of the brain is severed, or the brain removed, they are negative in strong and positive in weak light. The fact that severing the transverse commissure causes them to become positive to weak light indicates that the impulses normally cross over in the brain, but the fact that with the commissure severed or the brain removed they are still negative in strong light shows that under some conditions the impulses cross over in the ventral nerve cord.

If the conclusion that some photic impulses cross over in the transverse commissure is valid, it explains the fact that specimens with one connective cut are more strongly negative with illumination on the operated side than on the unoperated side, for the responses observed would then be produced as follows: 1) In specimens with one connective cut and with illumination on the operated side (fig. 8 A), all photic impulses which pass to the brain would cross over in the transverse commissure to the other side, enter the opposite connective and be blocked by the cut and rendered ineffective. In these specimens, therefore if the impulses relayed from the nerve cord to the muscles of the illuminated side are effective, a positive response would result.[3] 2) In specimens with one connective cut and with illumination on the operated side (fig. 8 B), photic impulses would pass from the cord to the brain but would be blocked by the cut in the connective on the illuminated side. But if some impulses from the sensi-

[3] This result would not be obtained in illumination strong enough to produce impulses which would cross over in the ventral nerve cord.

tive first and second segments passing unobstructed to the brain crossed over in the transverse commissure and passed down the opposite connective to the nerve cord and to the muscles of the shaded side of the body, and if these impulses were strong enough to oppose the tendency of the impulses in the ventral nerve cord to produce positive orientation, a negative response would result. These responses actually were obtained.

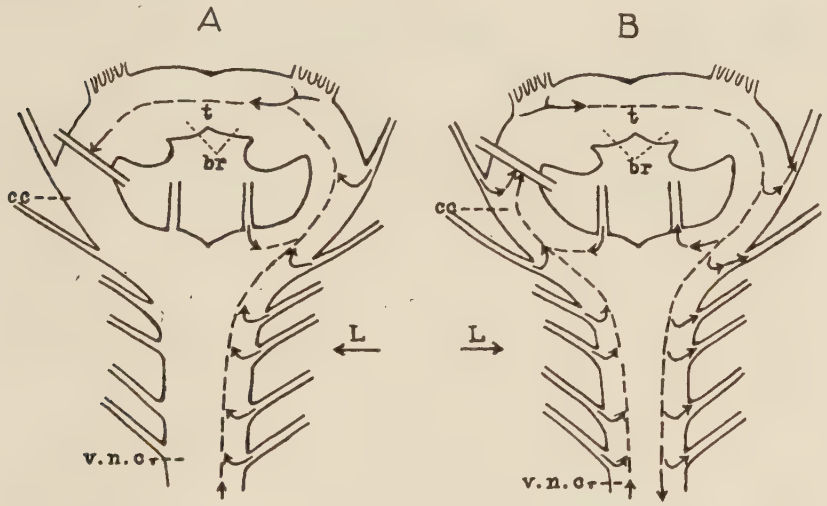

Fig. 8 Diagrams illustrating the course taken by nerve impulses produced by photic stimulation in worms with one circumpharyngeal connective severed. A, illuminated on the unoperated side; B, illuminated on the operated side; br, brain; cc, circumpharyngeal connective; t, transverse commissure of the brain; v.n.c., ventral nerve cord.

If these contentions are valid, the course of nerve impulses bringing about a negative response in normal specimens of Pheretima agrestis is as follows: Impulses from photic stimuli enter the central nervous system either at the brain, the circumpharyngeal connectives, or the ventral nerve cord. Strong impulses cross over directly in the ventral nerve cord as well as in the transverse commissure of the brain. Weak impulses either enter the brain directly through the cerebral nerves or are relayed anteriorly to the brain where they are

modified and cross over in the transverse commissure. After crossing over, impulses go to the muscles of the side opposite that which was illuminated causing them to contract, producing negative orientation.

If this is true, the fact that Pheretima agrestis is normally negative in all intensities of light to which it responds is probably explainable by the assumption that all intensities of light strong enough to produce orientation in light produce some impulses which are relayed to the brain, follow the path outlined above, and produce a negative response.

The fact that specimens with the brain removed or functionally impaired are positive in weak and negative in strong light indicates, furthermore, that there is a threshold intensity of light necessary to produce impulses which are strong enough to cross over in the ventral nerve cord.

SUMMARY

1. Pheretima agrestis is negative in all intensities of light to which it responds. It is more strongly negative in strong than in weak light.

2. Adaptation in overhead illumination raises the threshold intensity of light to which Pheretima agrestis responds, and decreases the negativity in weak light, but does not affect the negativity in moderately strong light.

3. As the temperature to which Pheretima agrestis is exposed increases, the degree of negativity increases to a maximum at 26° and then decreases slightly at 29°.

4. Excessive mechanical stimulation decreases the degree of negativity of Pheretima agrestis.

5. Excessive mechanical stimulation and high temperature probably decrease the negativity of Pheretima agrestis by producing responses which inhibit the response to light.

6. The order of sensitivity to light of the various regions of the body of Pheretima agrestis is as follows: anterior end > posterior end > middle portion of body; and the sensitivity in the anterior end is: second segment = third segment > prostomium > remainder of first segment > fourth segment.

7. All operations on the nervous system of the anterior end of the body decrease the degree of negativity to lateral illumination.

8. If the brain is removed, or if both circumpharyngeal connectives are cut, or if the transverse commissure of the brain is cut the decrease in negativity is so great that specimens become positive in weak light, but are still negative in moderately strong light.

9. If one circumpharyngeal connective is cut, illumination on the operated side produces a greater percentage of negative responses than illumination on the unoperated side.

10. Photonegative responses in normal specimens of Pheretima agrestis are probably brought about as follows: Impulses from photic stimuli travel along afferent nerves to the brain, the ventral nerve cord and the circumpharyngeal connectives. Strong impulses cross over directly in the transverse commissure of the brain and in the commissures of the ventral nerve cord. Weak impulses are relayed to the brain where, with impulses entering the brain from cerebral nerves, or from the circumpharyngeal connectives, they are probably altered and cross over in the transverse commissure. After crossing over, the impulses go to the muscles of the side opposite that which was illuminated causing them to contract, thus producing a negative response.

11. Photoreceptor cells were found in the epidermis of Pheretima agrestis.

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VITA

Charles DeWitt Howell was born in East Bangor, Pa., October 29, 1910. He received his elementary and secondary education in the public schools of Brooklyn, N. Y., and graduated from the New Utrecht High School in June, 1928.

In September, 1928, he matriculated at Oberlin College where he was Student Assistant in Biology during his senior year, and from which he received the A.B. degree on graduation in June, 1932. The following September he entered the Graduate School of the Johns Hopkins University as a student in zoology. For three years, 1932–1935, he was Graduate Assistant in Comparative Anatomy and Embryology at the latter university.

During the summers of 1934 and 1935 he was Research Assistant in the Conservation Department of the State of New York. In the year 1934–1935 he carried on several investigations. With Dr. W. G. Lynn, of the Johns Hopkins University, he published in 1935 and 1937 the results of one of these, an investigation of a cardiac inversion and translocation in a calf.

He was Instructor in Zoology in the University of Maryland from 1935–1937, and during these years and the summer of 1936 he carried on research under Prof. S. O. Mast, of the Johns Hopkins University, in connection with his doctor's dissertation. In 1936 he published a preliminary report of this work.

He was awarded the degree of Doctor of Philosophy from the Johns Hopkins University in June, 1937. The following summer he carried on work, on a scholarship from the University, as independent investigator at the Marine Biological Laboratory, Woods Hole, Mass. In the year 1937–1938 he was Professor of Biology at Elizabethtown College, Elizabethtown, Pa., and since then has been Assistant Professor of Biology at Middlebury College, Middlebury, Vt.